Chemistry 882

Lecture Notes 10

Welsky

An interesting and important question is understanding the contributing factors to why some proteins, RNA's, and synthetic polymers form compact folded structures in water => often the folded structure is important for function

One key point is that folding/formation of a single structure needs to compensate for large loss of conformational entropy

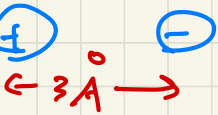
For a ~150 residue protein

$\Delta S_m \approx 1250 \ J/mole\text{-}K$ for refolding

$(300\ K)(\Delta S) \approx 400\ kJ/mole$

There are numerous electrostatic energies in a folded protein

"Salt bridges" are proximity of positive and negatively charged sidechains. However,



$$u \approx (CC)\left(\frac{e^2}{3\times10^{-10}\ m}\right)\left(\frac{1}{D}\right)$$

$$\approx \left(10^{10}\ \frac{J\cdot m}{C^2}\right)\left(\frac{3\times10^{-38}\ C^2}{3\times10^{-10}\ m}\right)\left(10^{-2}\right)$$

$$\approx 10^{-20}\ J\left(\frac{6\times10^{23}}{mole}\right) \approx 6\ \frac{kJ}{mole}$$

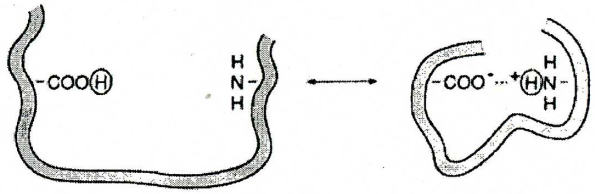
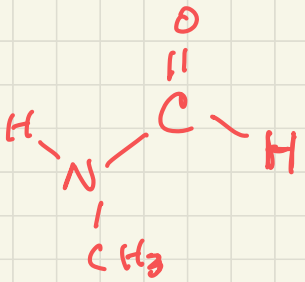


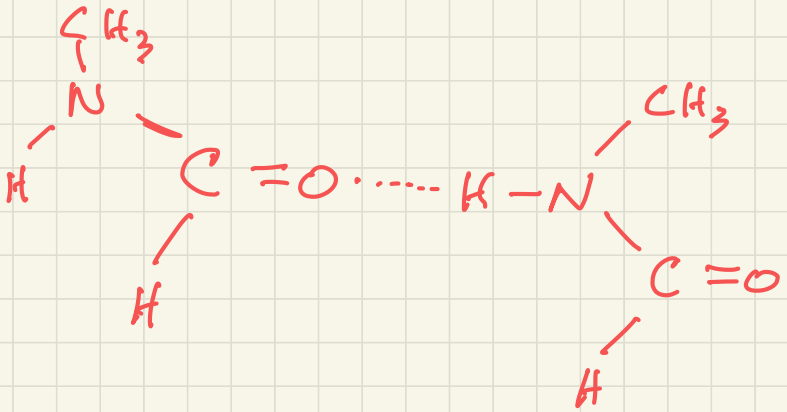
FIGURE 3: Early model in which protein folding was proposed to be driven by ion-paired hydrogen bonding among side chains (Mirsky & Pauling, 1936; Eyring & Stearn, 1939), shown by Jacobsen and Linderstrom-Lang (1949) to be inconsistent with partial molar volumes.

Hydrogen bonding is another important energy. An important question is whether hydrogen bonds in regular secondary structure are lower free energy than hydrogen bonds with water.

The model system to address this question is

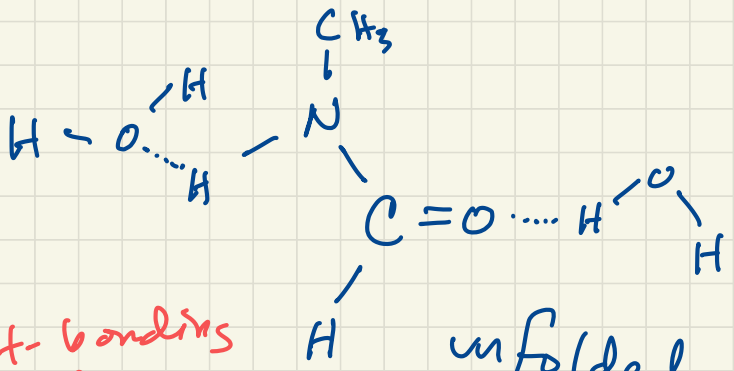


⇒ soluble in H_2O and CCl_4 (D close to Dprotein interior)



dimer

in CCl_4 ("folded protein")



monomer in H_2O

H-bonding in protein interior

unfolded protein

$$G_{dimer,\,CCl_4} - G_{monomer,\,H_2O} = -RT \ln \left\{ \frac{[dimer - CCl_4]}{[monomer - H_2O]^2} \right\}$$

$$= -RT \ln \left\{ \frac{[dimer - CCl_4]}{[monomer - CCl_4]^2} \right\} \left\{ \frac{[dimer - H_2O]}{[monomer - H_2O]^2} \right\}$$

experiment

Calculation

$$\left\{ \frac{[monomer - CCl_4]^2}{[dimer - H_2O]} \right\}$$

experiment $\approx 10^{kJ}/mole$

Another approach is additives to water that decrease H-bonding. However, additives like dioxane  and sodium dodecyl sulfate reduce T_m in addition to being poorer than H_2O at H-bonding.

Another possibility is that certain amino acids strongly favor a particular regular secondary structure and so a particular amino acid

sequence has the secondary structure encoded in it. However, there is not a strong amino acid / 2° structure correlation.

high α helix probability

low probability

high β sheet P

low β sheet P

Table 4.2 Propensities of Amino Acids to Form α-Helices $\langle P_\alpha \rangle$ and β-Sheets $\langle P_\beta \rangle$

α-Residues	$\langle P_\alpha \rangle$	α-Assignment	β-Residues	$\langle P_\beta \rangle$	β-Assignment
Glu	1.44 ± 0.06	H_α	Val	1.64 ± 0.07	H_β
Ala	1.39 ± 0.05	H_α	Ile	1.57 ± 0.08	H_β
Met	1.32 ± 0.11	H_α	Thr	1.33 ± 0.07	h_β
Leu	1.30 ± 0.05	H_α	Tyr	1.31 ± 0.09	h_β
Lys	1.21 ± 0.05	h_α	Trp	1.24 ± 0.14	h_β
His	1.12 ± 0.08	h_α	Phe	1.23 ± 0.09	h_β
Gln	1.12 ± 0.07	h_α	Leu	1.17 ± 0.06	h_β
Phe	1.11 ± 0.07	h_α	Cys	1.07 ± 0.12	h_β
Asp	1.06 ± 0.06	h_α	Met	1.01 ± 0.13	I_β
Trp	1.03 ± 0.10	I_α	Gln	1.00 ± 0.09	I_β
Arg	1.00 ± 0.07	I_α	Ser	0.94 ± 0.06	i_β
Ile	0.99 ± 0.06	i_α	Arg	0.94 ± 0.09	i_β
Val	0.97 ± 0.05	i_α	Gly	0.87 ± 0.05	i_β
Cys	0.95 ± 0.09	i_α	His	0.83 ± 0.09	i_β
Thr	0.78 ± 0.05	i_α	Ala	0.79 ± 0.05	i_β
Asn	0.78 ± 0.06	i_α	Lys	0.73 ± 0.06	b_β
Tyr	0.73 ± 0.06	b_α	Asp	0.66 ± 0.06	b_β
Ser	0.72 ± 0.04	b_α	Asn	0.66 ± 0.06	b_β
Gly	0.63 ± 0.04	B_α	Pro	0.62 ± 0.07	B_β
Pro	0.55 ± 0.05	B_α	Glu	0.51 ± 0.06	B_β

Listed are values compiled from the crystal structures of 64 proteins, and the assignments as former (*H* and *h*), indifferent (*I* and *i*) and breakers (*b* and *B*) for each type of structure.

From P. Y. Chou (1989), in *Prediction of Protein Structure and the Principles of Protein Conformation*, ed. G. D. Fasman, 549–586, Plenum Press, New York.

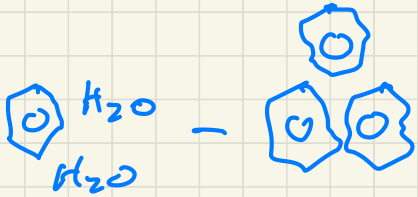
Most amino acid types are found in both α helix and β sheet secondary structures in folded proteins

There are several lines of evidence that the hydrophobic effect contributes to the folded protein stability near ambient temperature ⇒

$\Delta C_p \approx 8000$ J/mole-K for 150-residue protein

unfolded - folded

$\Delta C_p \approx 350$ J/mole-K



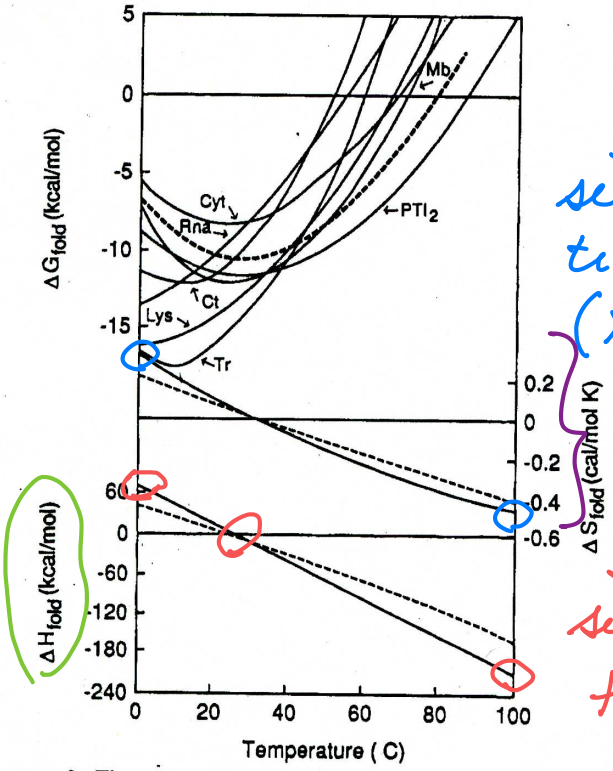


FIGURE 8: Thermal stabilities of proteins. Experimental data for free energies, enthalpies, and entropies of folding taken from Privalov (1979) and Privalov and Kechinashvili (1974) (—). Theoretical predictions are from Dill et al. (1989) (--).

similar to transfer entropy (x 30)

0.8 kcal/mole-K ≈ 3000 J/mole-K

kcal/mol-K

similar to transfer enthalpy of ⬡ to H_2O (x 30)

$H_{fold} - H_{unfold}$

60 kcal/mole ≈ 250 KJ/mole

1 kcal x 4 KJ

Some proteins exhibit cold denaturation

Can ΔC_p ($C_{p,unfolded} - C_{p,folded}$) explain cold denaturation?

$\Delta G_{unfold} = G_{unfolded} - G_{folded}$

$= \Delta H_{unfold} - T\Delta S_{unfold}$

T_0 = zero-crossing temperature for ΔH_{unfold} and ΔS_{unfold}

$\approx 300\ K$

haven't included $\Delta H_m - T\Delta S_m$

$\Delta T = T - T_0$

$$\Delta G = (\Delta C_p)(\Delta T) - (T)(\Delta C_p)\ln\left(\frac{T}{T_0}\right)$$

$$= (\Delta c_p)(\Delta T) - (T_0 + \Delta T)(\Delta c_p) \ln\left\{1 + \frac{\Delta T}{T_0}\right\}$$

$$\approx (\Delta c_p)(\Delta T) - (T_0 + \Delta T)(\Delta c_p)\left(\frac{\Delta T}{T_0}\right) \quad \frac{\Delta T}{T_0} \ll 1$$

$$= \cancel{(\Delta c_p)(\Delta T)} - \cancel{(\Delta c_p)(\Delta T)} - \frac{(\Delta c_p)(\Delta T)^2}{T_0}$$

$$= -\frac{(\Delta c_p)(\Delta T)^2}{T_0} \Rightarrow |\Delta T| \uparrow \quad \Delta G_{unfold} \downarrow$$

Add back in $\Delta H_m - T\Delta S_m$ contribution

$$\Delta H_m - T\Delta S_m = \Delta H_m - T\left(\frac{\Delta H_m}{T_m}\right)$$

approximate as T_0

$$\approx \Delta H_m - \left(\frac{T}{T_0}\right)(\Delta H_m)$$

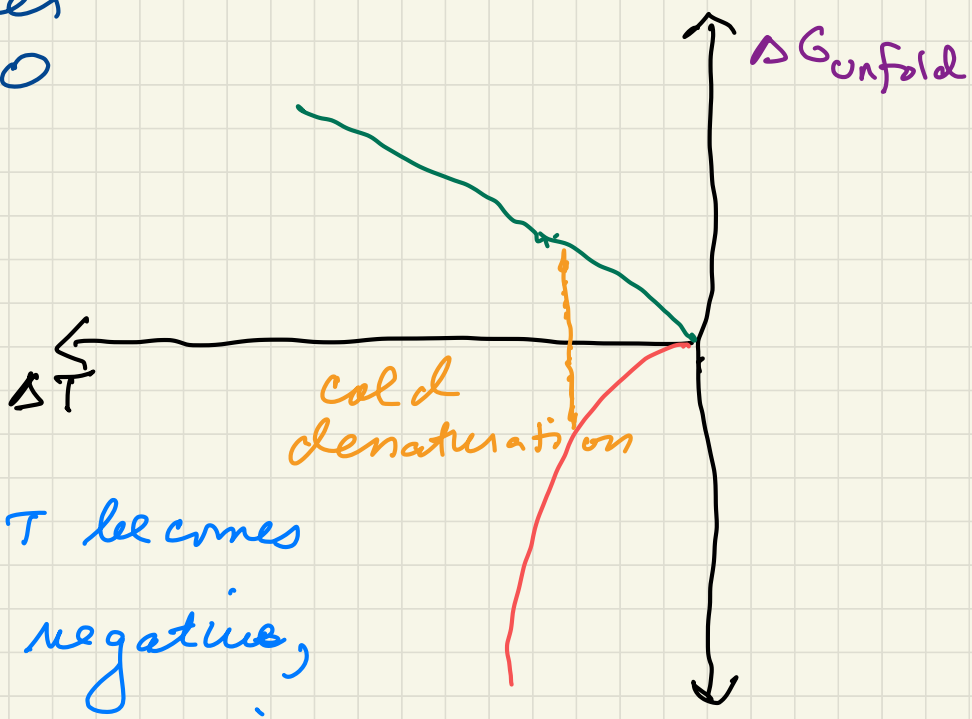
$$= \Delta H_m\left(1 - \frac{T}{T_0}\right) = \Delta H_m\left(\frac{T_0 - (T_0 + \Delta T)}{T_0}\right)$$

$$= (\Delta H_m)\left(-\frac{\Delta T}{T_0}\right) \Rightarrow \Delta T < 0, \text{ term} > 0 \quad \Delta G_{unfold} \uparrow$$

Total ΔG_{unfold}

$$\frac{1}{T_0}\left\{(\Delta H_m)(-\Delta T) - (\Delta C_p)(\Delta T)^2\right\}$$

Consider $\Delta T < 0$



As ΔT becomes more negative, ΔG_{unfold} will eventually become $< 0 \Rightarrow$ cold denaturation

Additives which decrease (increase) hydrocarbon solubility in H_2O increase (decrease) protein T_m

decreases hydrocarbon solubility in H_2O

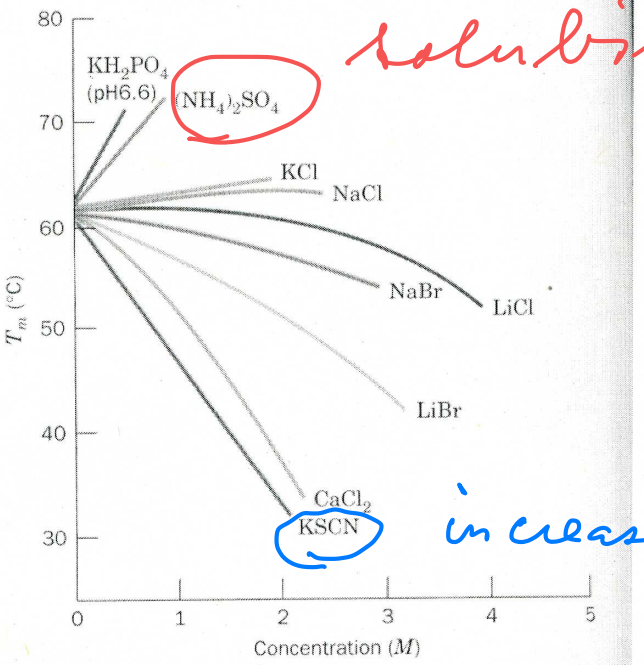
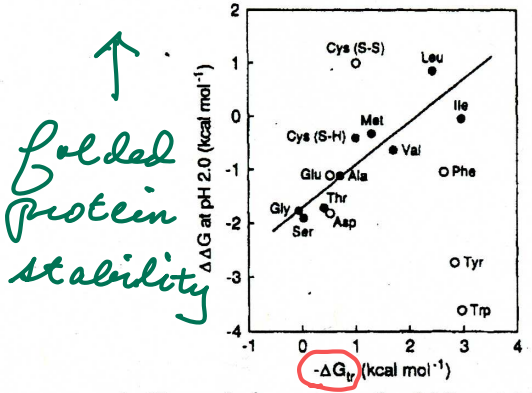


FIGURE 8-62 **Melting temperature of RNase A as a function of the concentrations of various salts.** All solutions also contained 0.15*M* KCl and 0.013*M* sodium cacodylate buffer, pH 7. [After von Hippel, P.J. and Wong, K.Y., *J. Biol. Chem.* **10,** 3913 (1965).]

increases hydrocarbon solubility in H_2O



↑ Folded protein stability

FIGURE 4: Change in free energy of unfolding, $\Delta\Delta G$, of mutant T4 lysozymes at position 3 (wild type is Ile) by substitution of other residues, compared to the corresponding free energy of transfer from water to ethanol. ΔG_{tr}. Reprinted with permission from Matsumura, M., Becktel, W. J., & Matthews, B. W. (1988) *Nature 334*, 406. Copyright (1988) Macmillan Magazines Limited.

ΔG transfer of amino acid from EtOH → H_2O hydrophobic effect →

Given the hydrophobic effect, why might regular secondary structure form?